A Short Introduction to Chemical Biology and Medicinal Chemistry

Part II Ben Davis [Ben.Davis@chem.ox.ac.uk] – 3 Lectures - Enzymes and Their Uses

Prior Knowledge Required

1. 1st year Biological Chemistry course: enzyme catalysis principles; Michaelis-Menten kinetics basics of protein structure; rough idea of transcription/translation/DNA→mRNA→protein; one letter and three letter codes for amino acids
2. 1st year Stereochemistry: resolution techniques
3. 2nd year Organic Reaction Mechanisms II: enzyme mechanism aspects

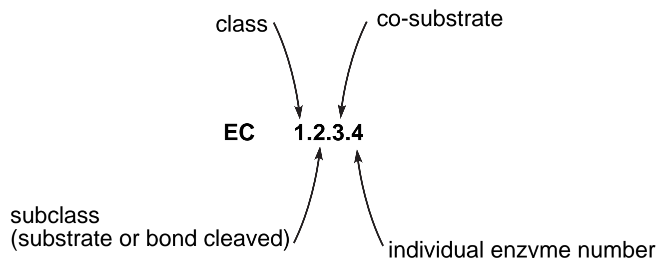
Books:

"Enzyme Structure and Mechanism" Fersht;
"An Introduction to Biotransformations in Organic Chemistry" Hanson;
"Biotransformations in Organic Chemistry" Faber;
"Biochemistry and Molecular Biology" Elliott & Elliott
OCP 98 Foundations of Chemical Biology
OCP 99 Carbohydrate Chemistry

Topics to be Covered

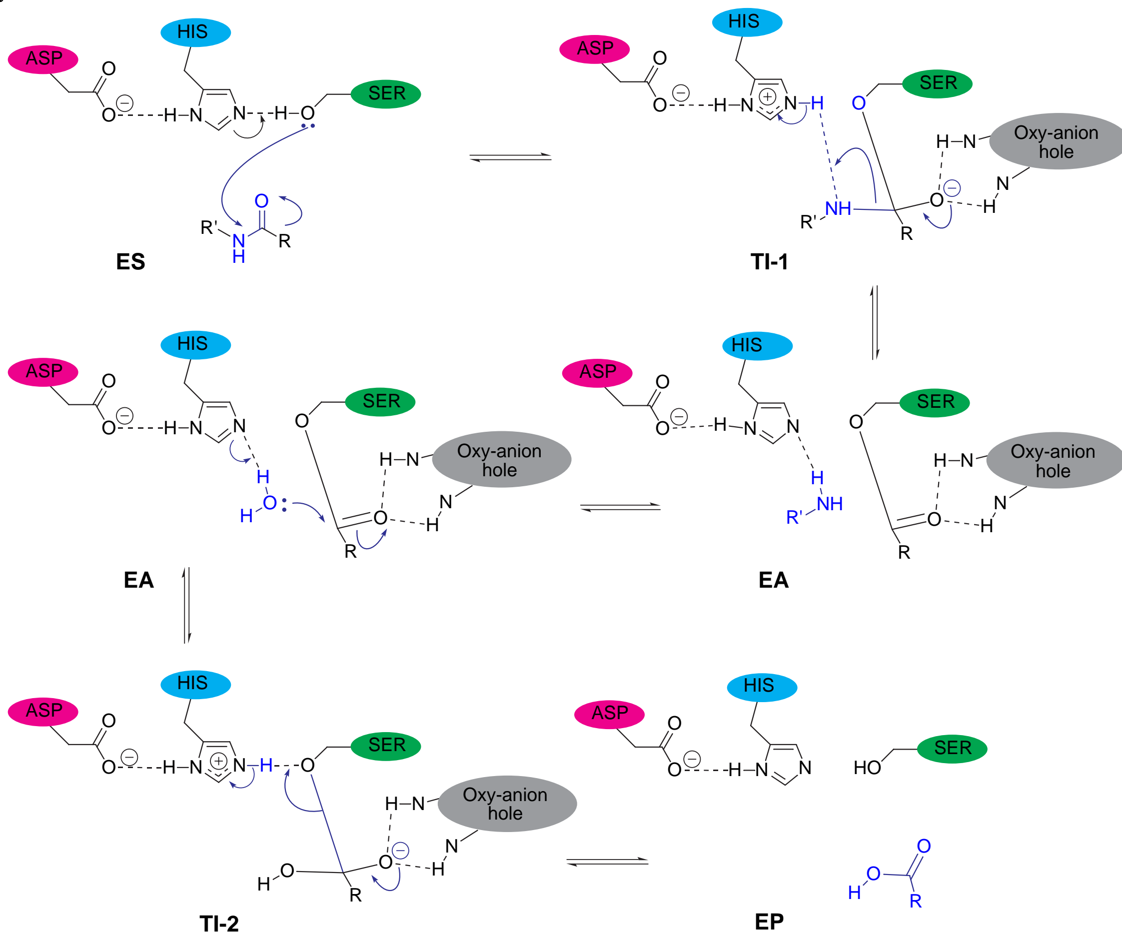
- Enzymes in Synthesis - prejudices; advantages & disadvantages; Enzyme Classifications and Reactions;
- Serine Hydrolases are Really Acyl Transferases; Common Mechanisms and Diverse Mechanisms e.g. Serine Proteases, Metalloproteases & Carboxyproteases; Ribosomal peptide synthesis;
- Acyl transferases in synthesis; regioselective transformations; stereoselective transformations; resolution techniques; desymmetrization;
- Peptide ligation; protein ligation (enzymatic & native chemical); inteins.
- Carbohydrate Processing Enzymes: Glycosidases and Glycosyltransferases
- Protein Engineering; techniques and results; mutagenesis & chemical modification; introduction of non-coded amino acids into proteins; examining the effects; Creating new catalysts: *de novo* enzymes, polyamino acid catalysts, engineered enzymes, catalytic antibodies; novel subtilisins.
- Sample Exam Questions

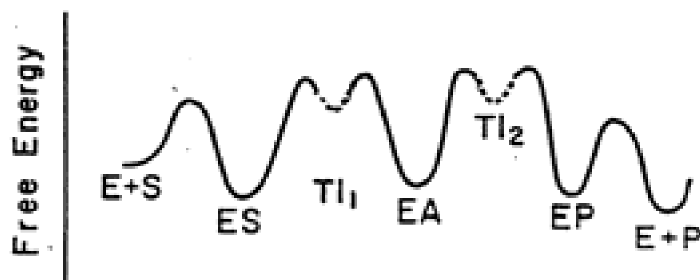
Enzyme classification
Enzyme commission 1955 (IUPAC)
EC 1.2.3.4
~20,000? exist
Online directory http://www.expasy.ch/enzyme/



Class	Reaction Type	Number		Usage
		Classified	Available	
1. Oxidoreductases	Redox: C-H, C-C, C=C oxygenation; (de)hydrogenases	~1000	~100	25%
2. Transferases	Transfer acyl, sugar, phosphoryl, methyl	~1000	~100	10%
3. Hydrolases	Hydolyse/form esters, amides, lactones, lactams, epoxides, nitriles, anhydrides, glycosides	~1000	~300	55%
4. Lyases	Addition/elimination to C=X (X = C, N, O)	~300	~50	5%
5. Isomerases	Racemization, epimerization	~150	~10	3%
6. Ligases	Formation/cleavage of C-X (O, S, N, C)	~100	~10	2%

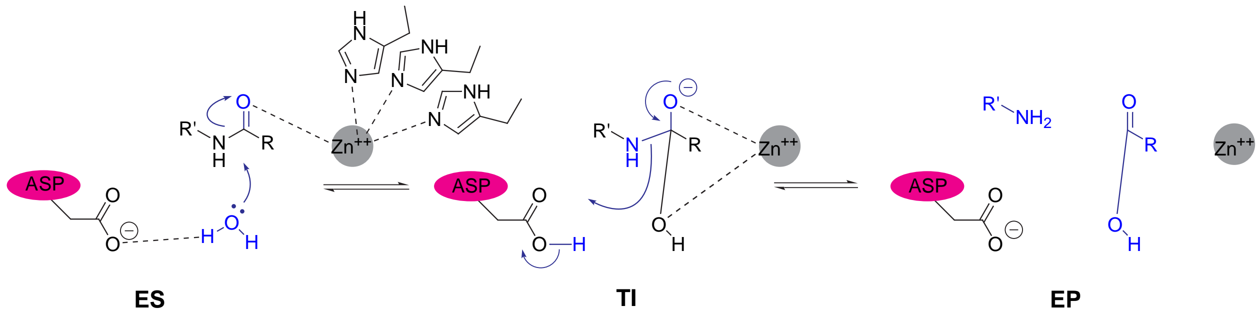
Serine Hydrolases

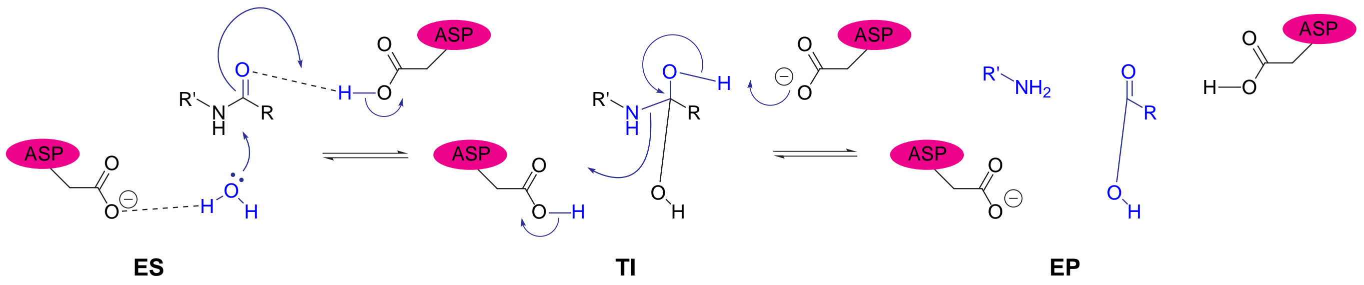




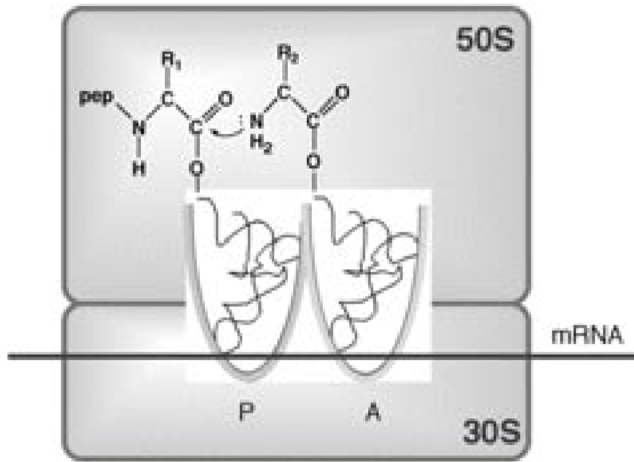
A representation of the expected free energy diagram for serine proteinase catalysis. From evolutionary principles the free energies of all the transition states are expected to be similar, and the energies of all the intermediates are anticipated to be similar

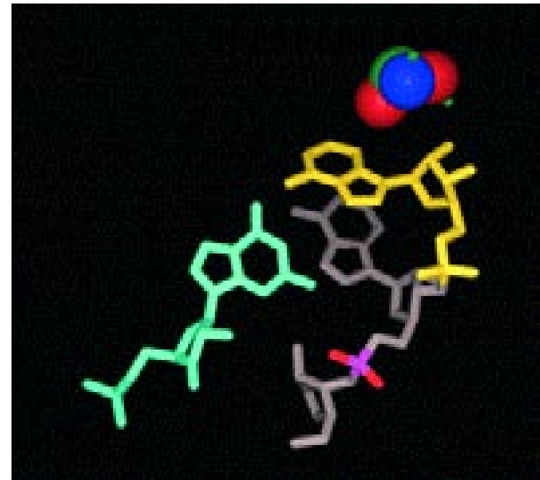
Metallo/Aspartylproteases

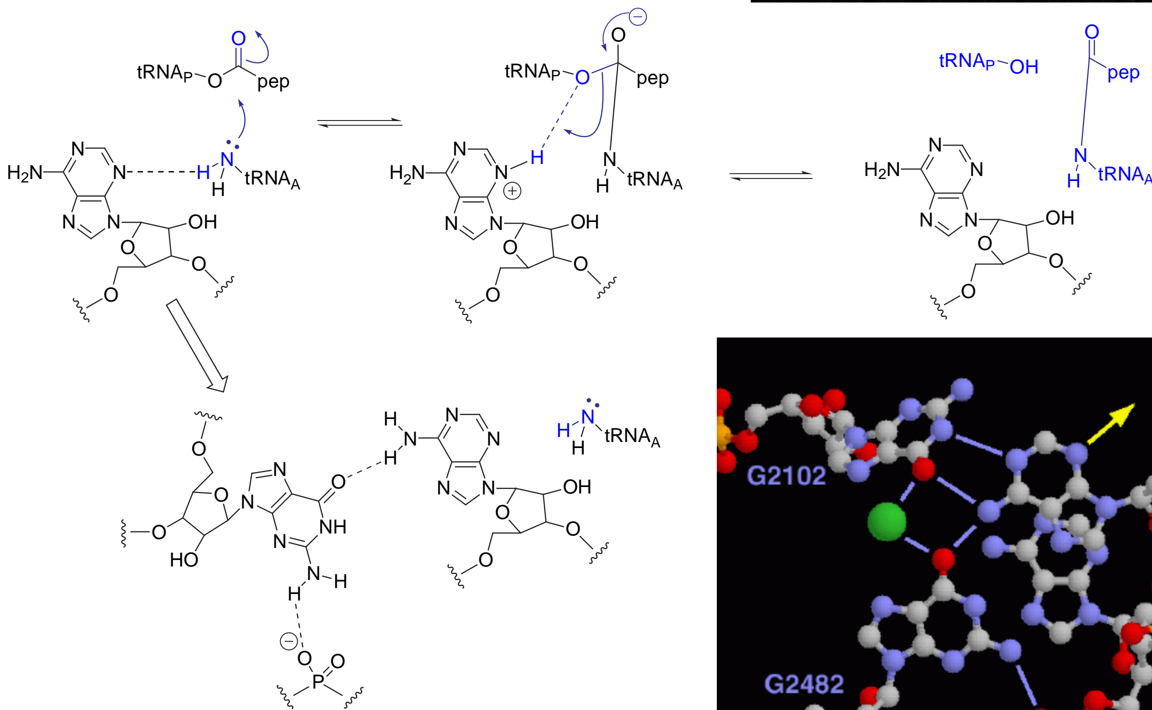


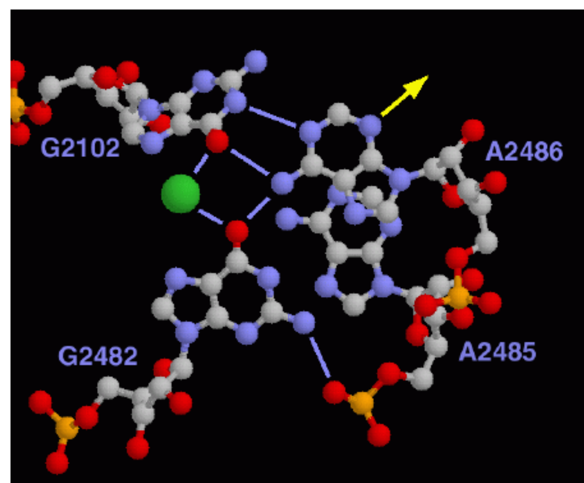


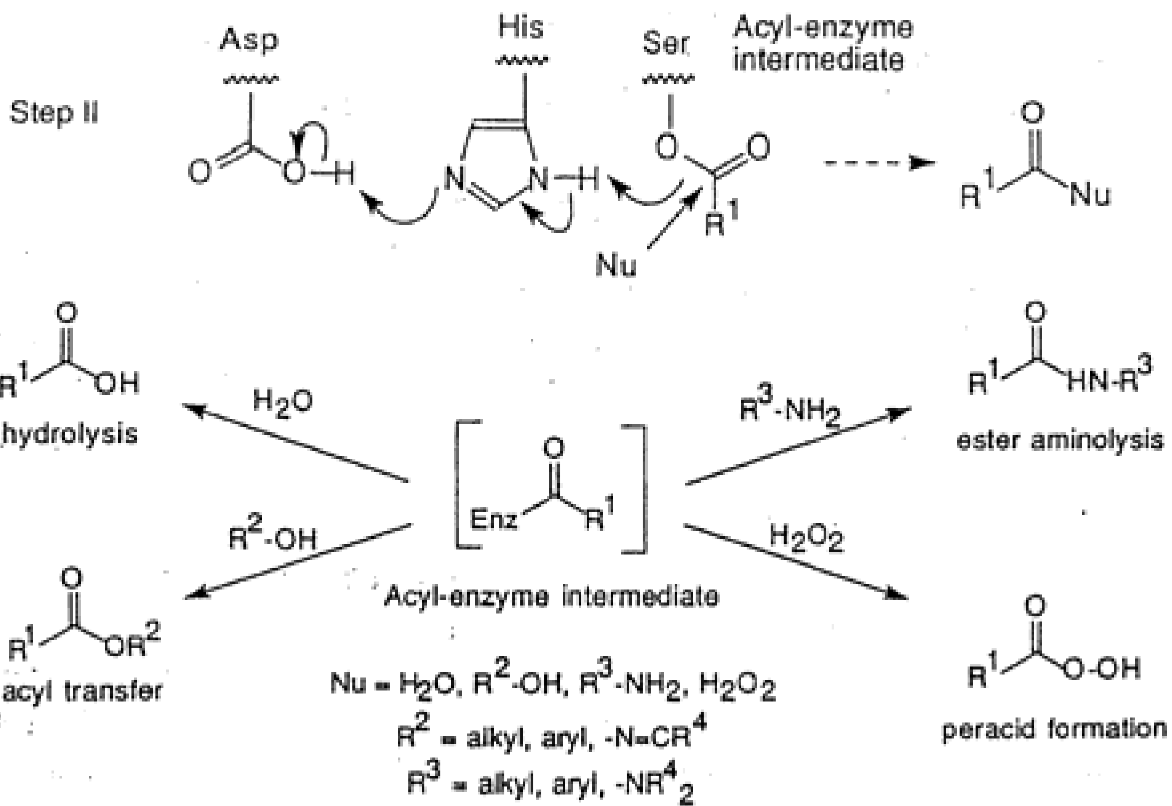
The Ribosome











α-CT, H_2O / PPL, H_2O

PPL, tBuOH

α-CT, pyridine

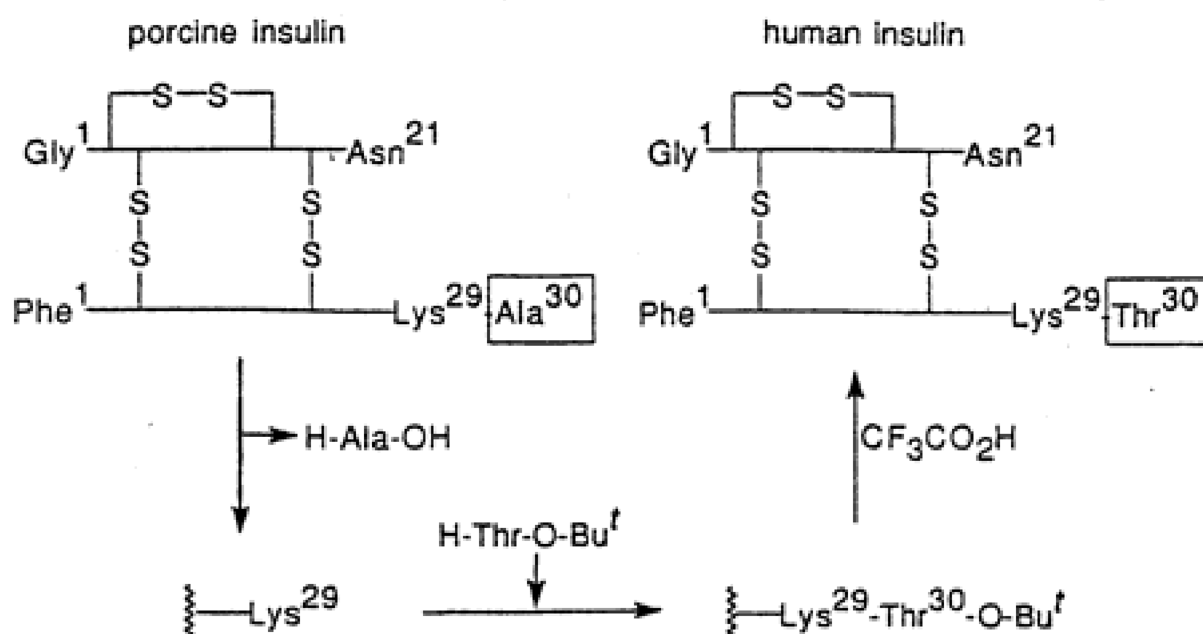
PPL, THF

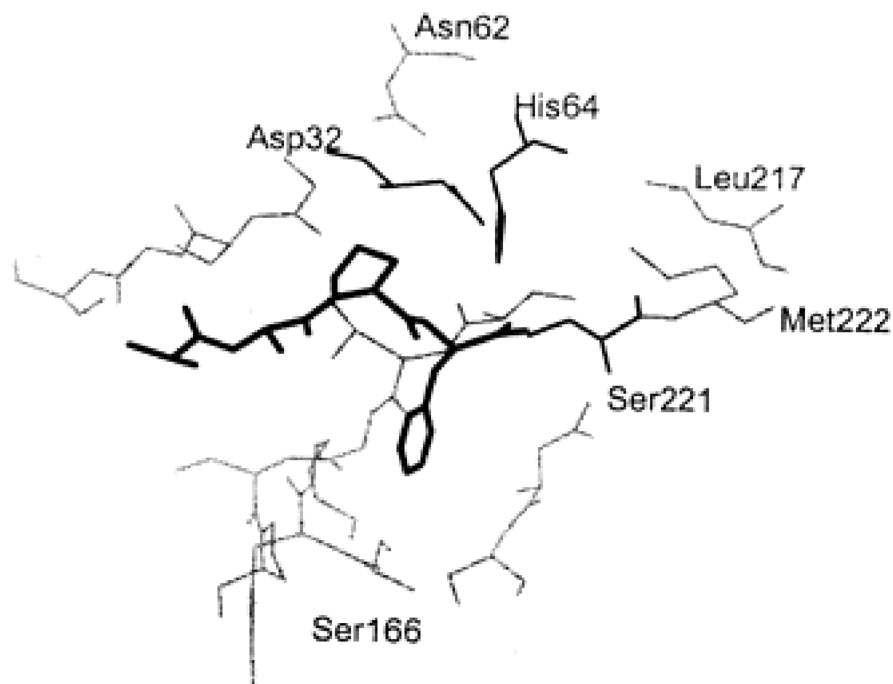
α-CT, H_2O / PLE, H_2O

Common proteases and their preferred cleavage sites.

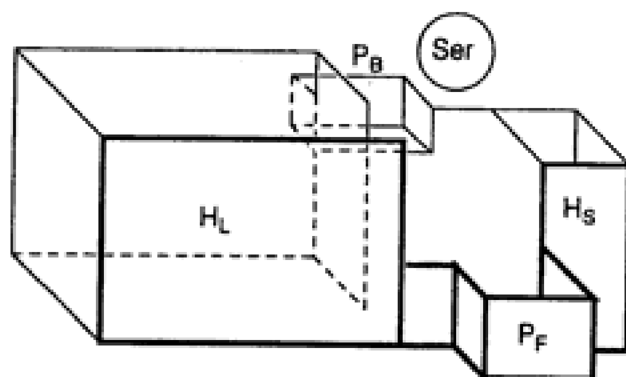
Protease	Type	Preferred Cleavage Sites
α-chymotrypsin and subtilisins	Ser	-Trp(Tyr,Phe,Leu,Met) ↓ Xaa-
elastase	Ser	-Ala(Ser,Met,Phe) ↓ Xaa-
pepsin	Asp	-Phe(Tyr,Leu) ↓ Leu(Phe)-
thermolysin	metallo	-Phe(Gly,Asp,Leu) ↓ Leu(Phe)-
papain	Cys	-Phe(Leu,Val)-Xaa ↓ Xaa-
trypsin	Ser	-Arg(Lys) ↓ Xaa-
clostripain	Cys	-Arg ↓ Xaa-
endoprotease Lys-C (*Achromobacter*)	Ser	-Lys ↓ Xaa-
endoprotease Glu-C (V8 protease)	Ser	-Glu (Asp) ↓ Xaa-
carboxypeptidase Y	Ser	-Xaa ↓ Xaa-OH
carboxypeptidase B	metallo	-Xaa ↓ [Arg,Lys]-OH
carboxypeptidase A	metallo	-Xaa ↓ [Asp,Glu,Phe,Leu]-OH
aminopeptidase M	metallo	H_2N-Xaa ↓ Xaa-
pyroglutamate-aminopeptidase	Cys	pGlu ↓ Xaa-
cathepsin C	Cys	H_2N-Xaa-Xaa ↓ Xaa-
proline iminopeptidase	Ser	Pro ↓ Xaa-

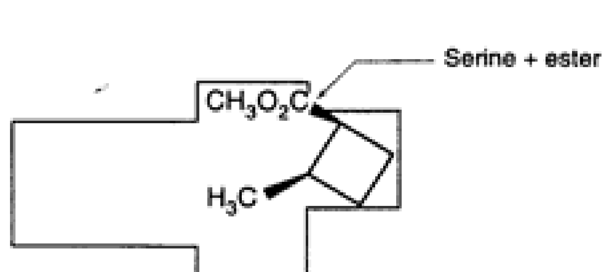
Enzymatic conversion of porcine into human insulin.

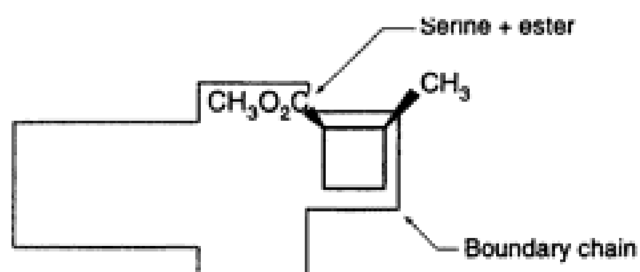


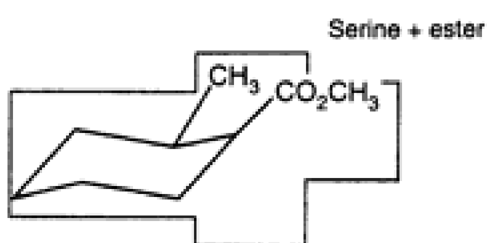


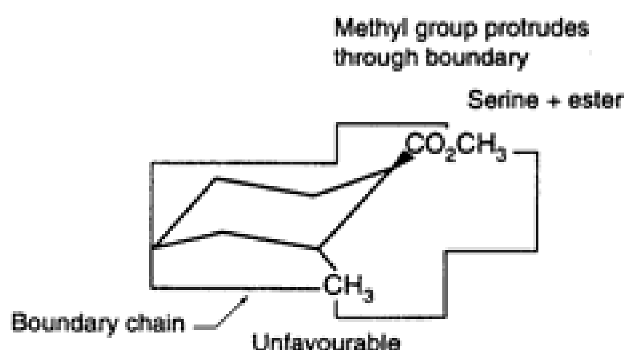
Active-site model for PLE.

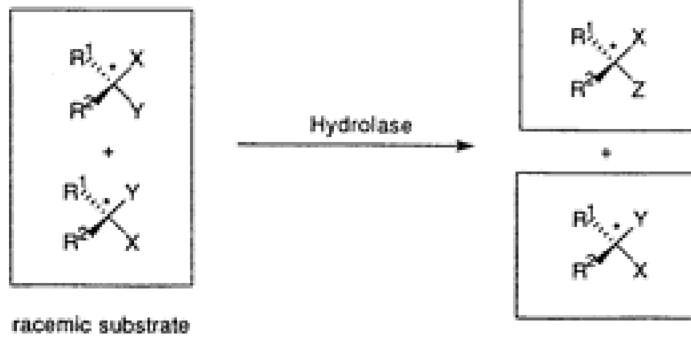


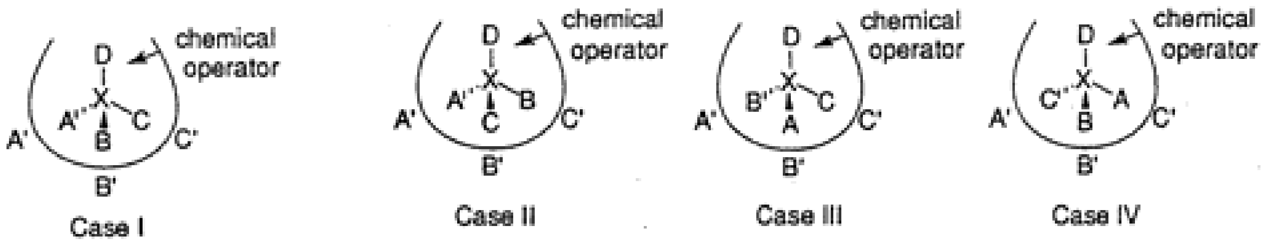


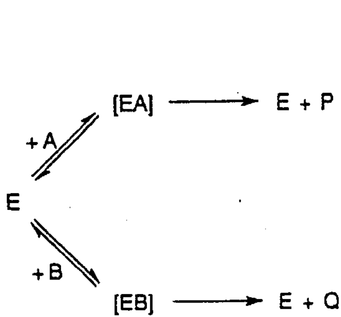


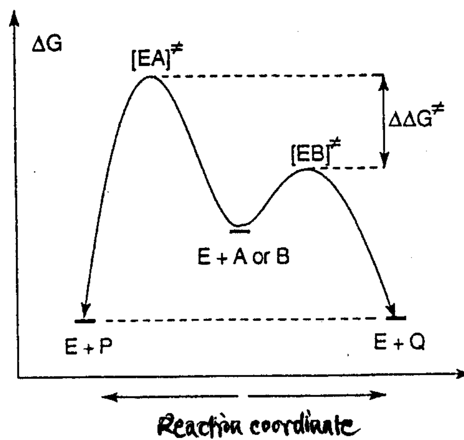






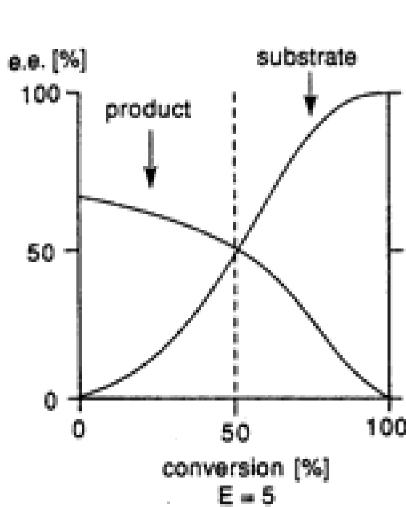


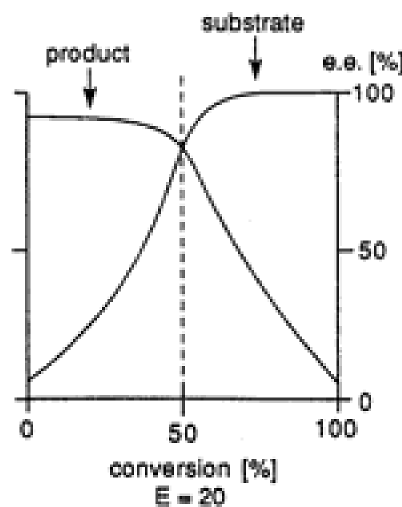


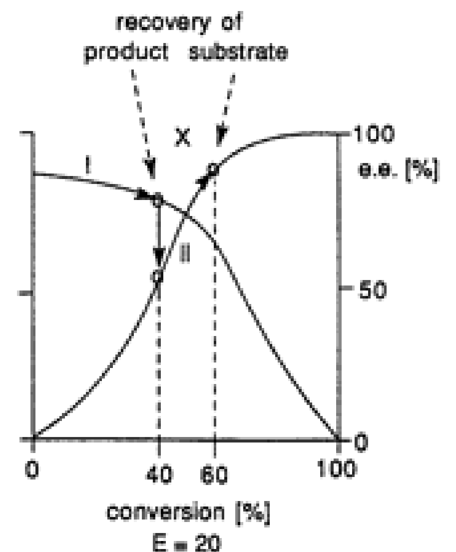


$\Delta\Delta G^{\neq}$ (kcal/mol)	e.e. (%)
0.118	10
0.651	50
1.74	90
2.17	95
3.14	99
4.50	99.9

$$\text{e.e. (\%)} = \frac{P - Q}{P + Q} \times 100$$







rate of reaction $v = [E]_0.k_{cat}.[S] / (K_M + [S])$ *Michaelis-Menten equation*

$\therefore$ for a kinetic resolution $v_A / v_B = (k_{cat}/K_M)_A[A] / (k_{cat}/K_M)_B[B]$

e.e. = $[(P - Q) / (P + Q)] * 100 = [(v_A / v_B - 1) / (v_A / v_B + 1)] * 100$

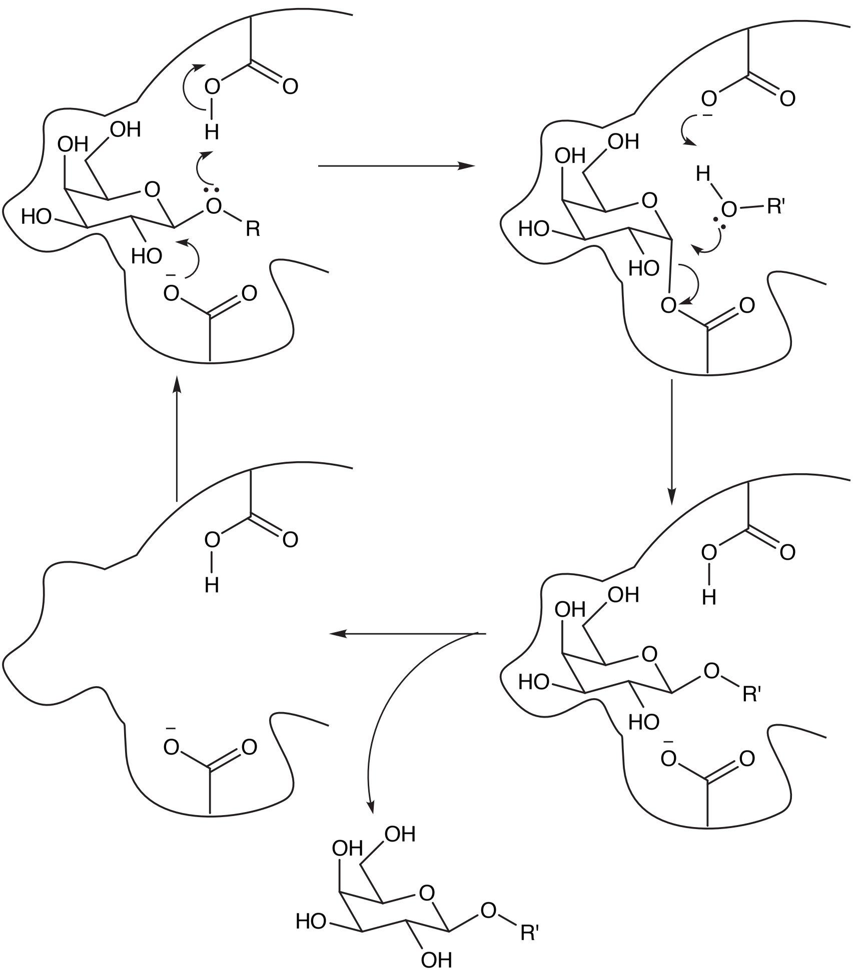
$\therefore$ the e.e. changes as the reaction does i.e. the stereoselectivity is dependant on conversion (c)

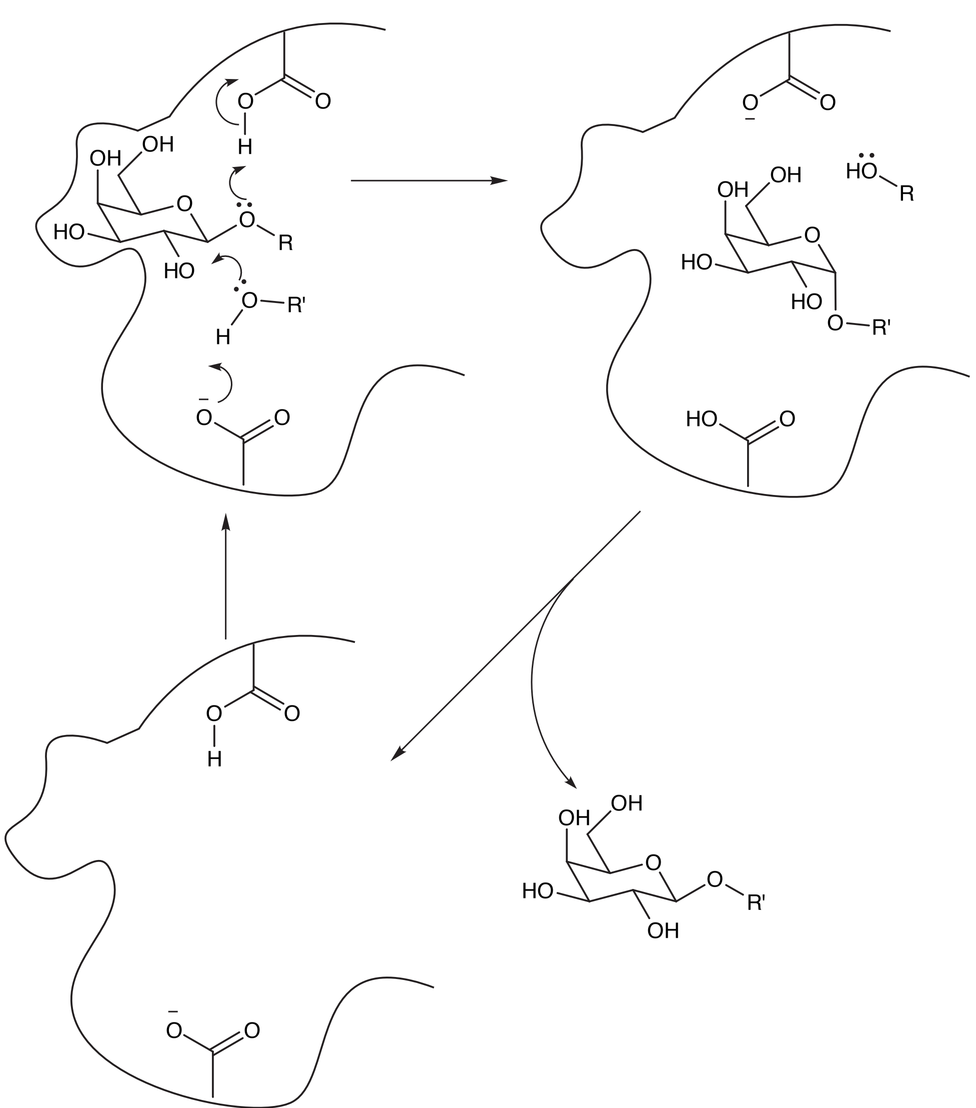
We need an independent measure of stereoselectivity in kinetic resolutions

$\mathbf{E} = (k_{cat}/K_M)_A / (k_{cat}/K_M)_B = \ln [1\text{-}c(1 + ee_P)] / \ln[(1\text{-}c(1 - ee_P)]$

normally we look for $E \geq 20$

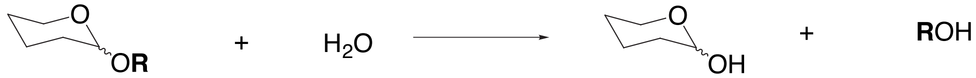
(PS You don't need to be able to derive these)





GLYCOSIDASES

Naturally, they catalyze the hydrolysis of the glycosidic bond, i.e., the split glycosides

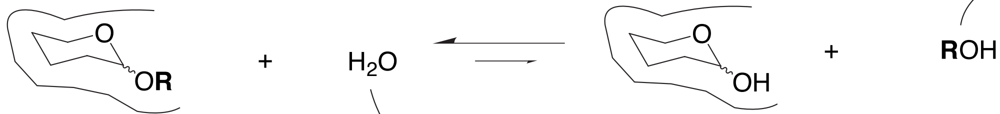


To make glycosidic bonds i.e., to make

there are TWO tactics:

1. Reverse the Equilibrium

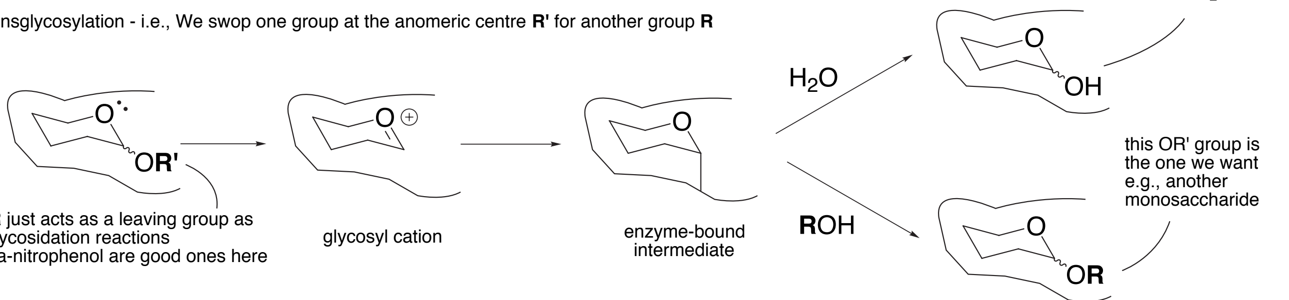
lots of this to drive towards other side



remove this to drive equilibrium overto the left

2. Transglycosylation - i.e., We swop one group at the anomeric centre **R'** for another group **R**

Prevent formation of this by trying to exclude H_2O



this OR' group is the one we want e.g., another monosaccharide

This OR just acts as a leaving group as for all glycosidation reactions e.g, para-nitrophenol are good ones here

glycosyl cation

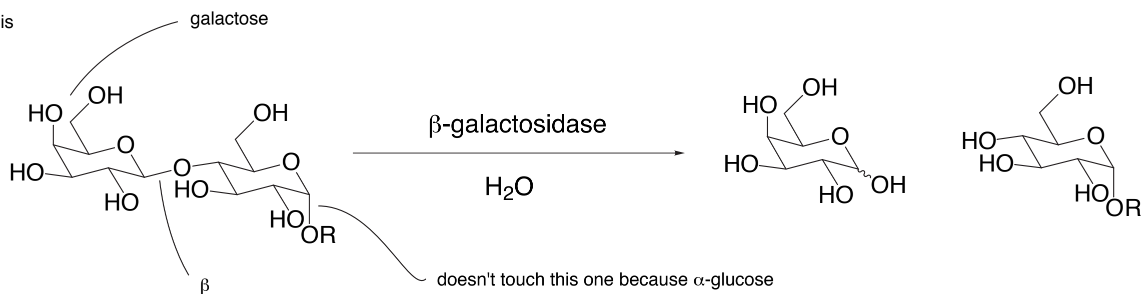
enzyme-bound intermediate

N.B. This is also the mechanism for the equilibrium shown above at the top and in **1.**

EXAMPLES OF GLYCOSIDASE REACTIONS

Hydrolysis

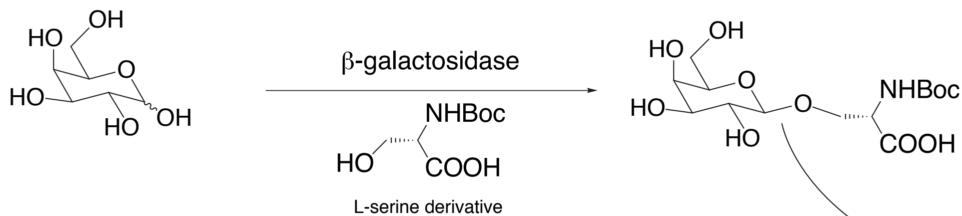
galactose



β

doesn't touch this one because α-glucose

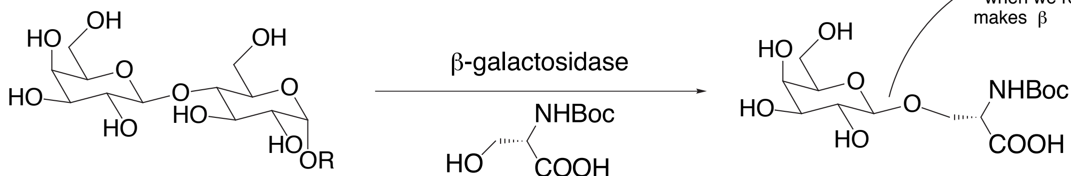
Equilibrium Reversal



L-serine derivative

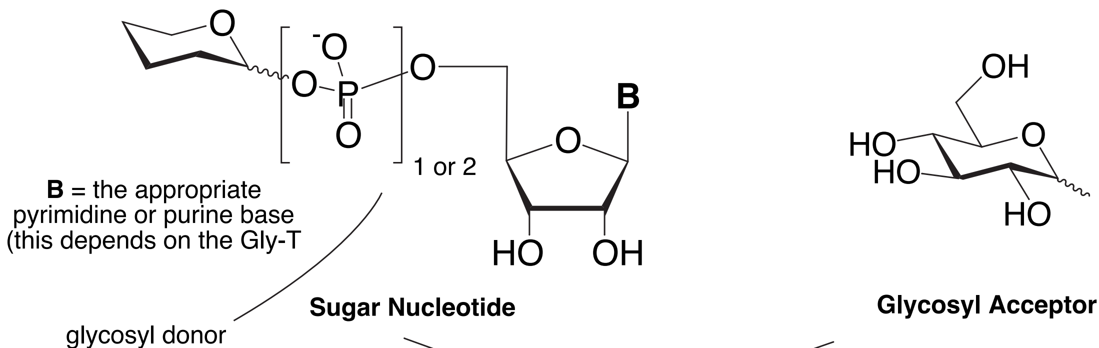
Because normally it only cleaves β - when we reverse things it only makes β

Transglycosylation



GLYCOSYLTRANSFERASES

Leloir Type



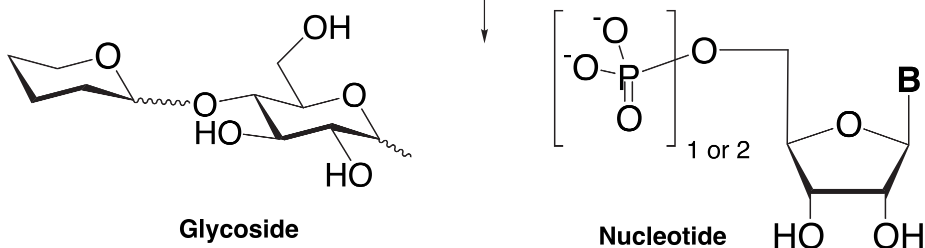
B = the appropriate pyrimidine or purine base (this depends on the Gly-T

glycosyl donor

Sugar Nucleotide

Glycosyl Acceptor

Glycosyltransferase

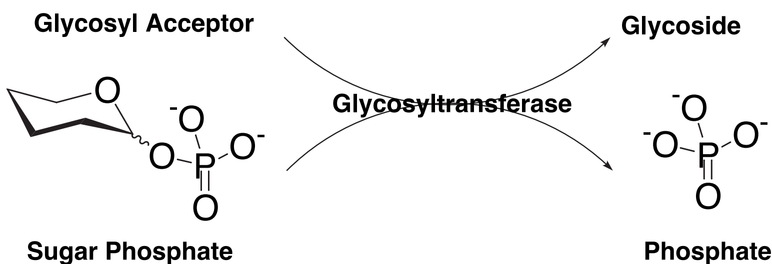


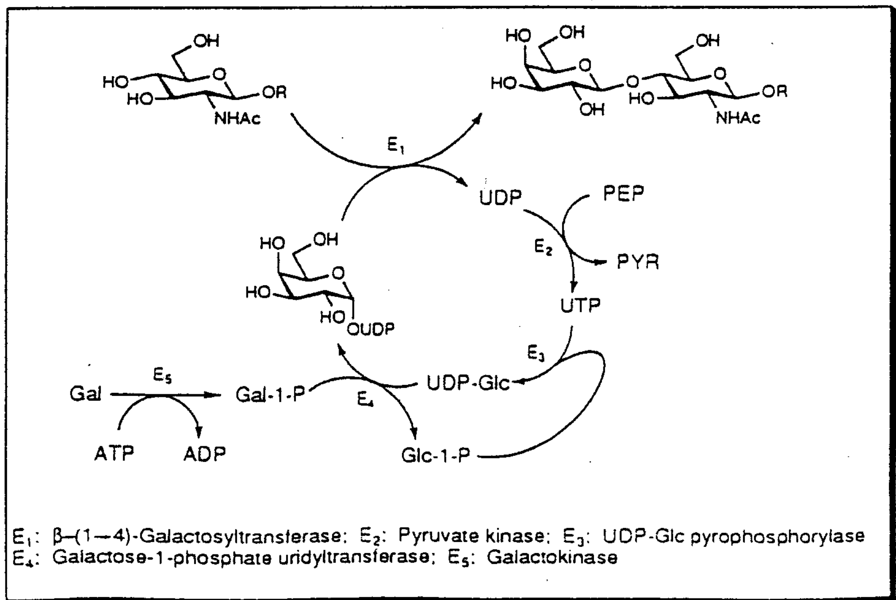
Glycoside

Nucleotide

Non-Leloir Type

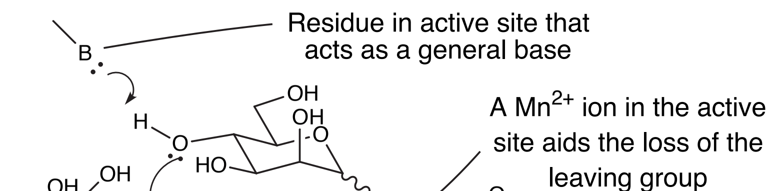
Glycosyl donor is a Sugar Phosphate instead and so the leaving group is just phosphate ion



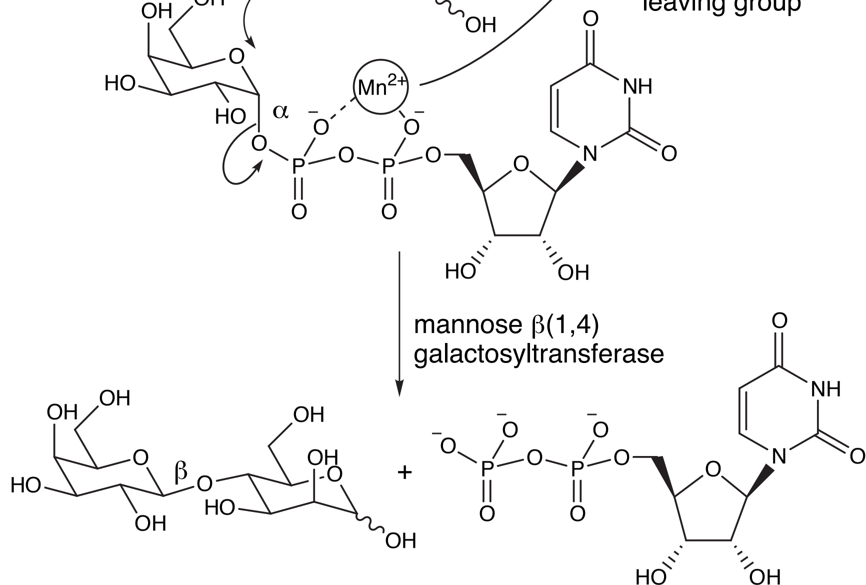


Residue in active site that acts as a general base

A Mn^{2+} ion in the active site aids the loss of the leaving group

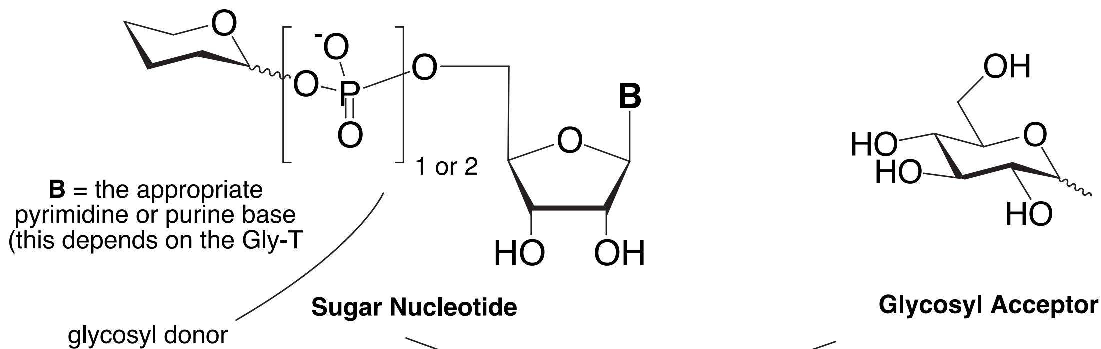


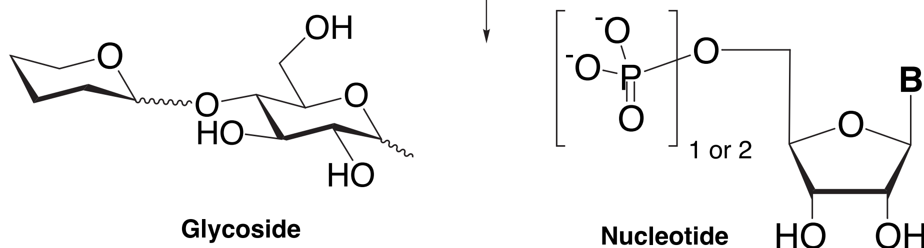
mannose β(1,4) galactosyltransferase



GLYCOSYLTRANSFERASES

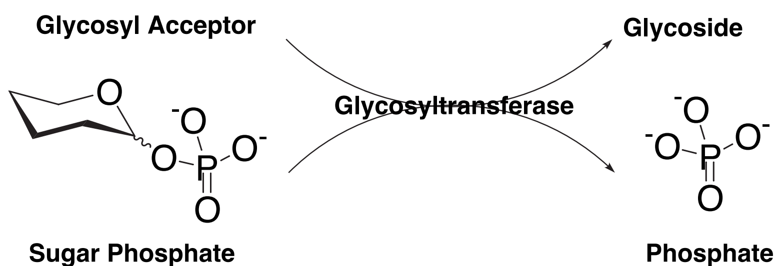
Leloir Type

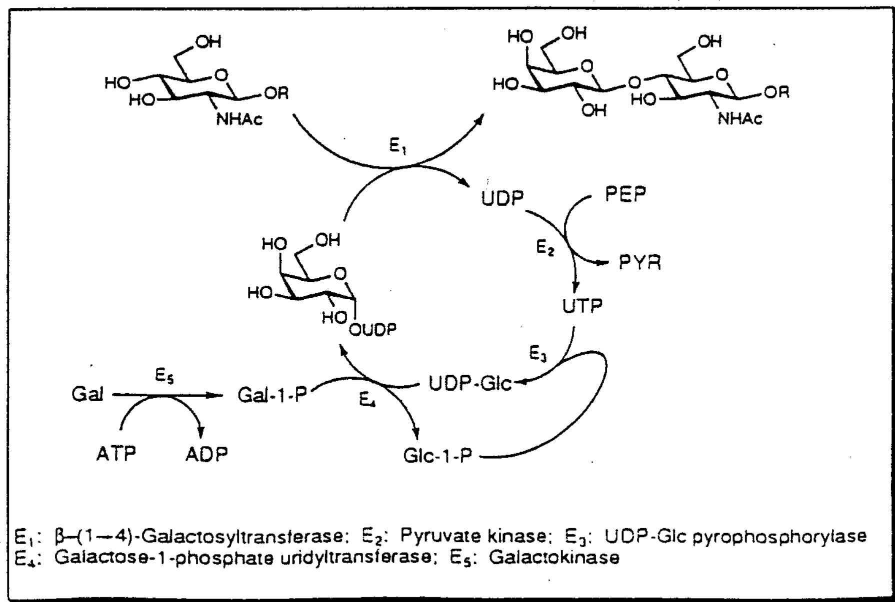




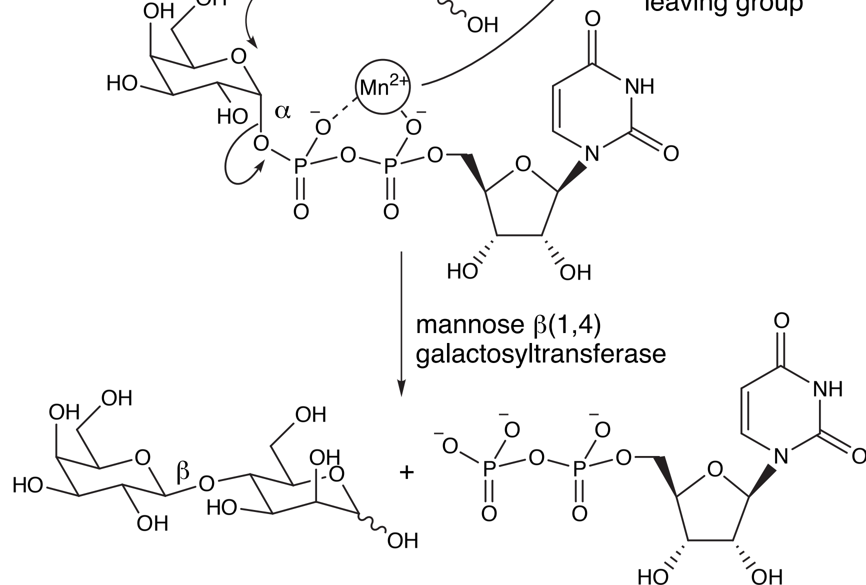
Non-Leloir Type

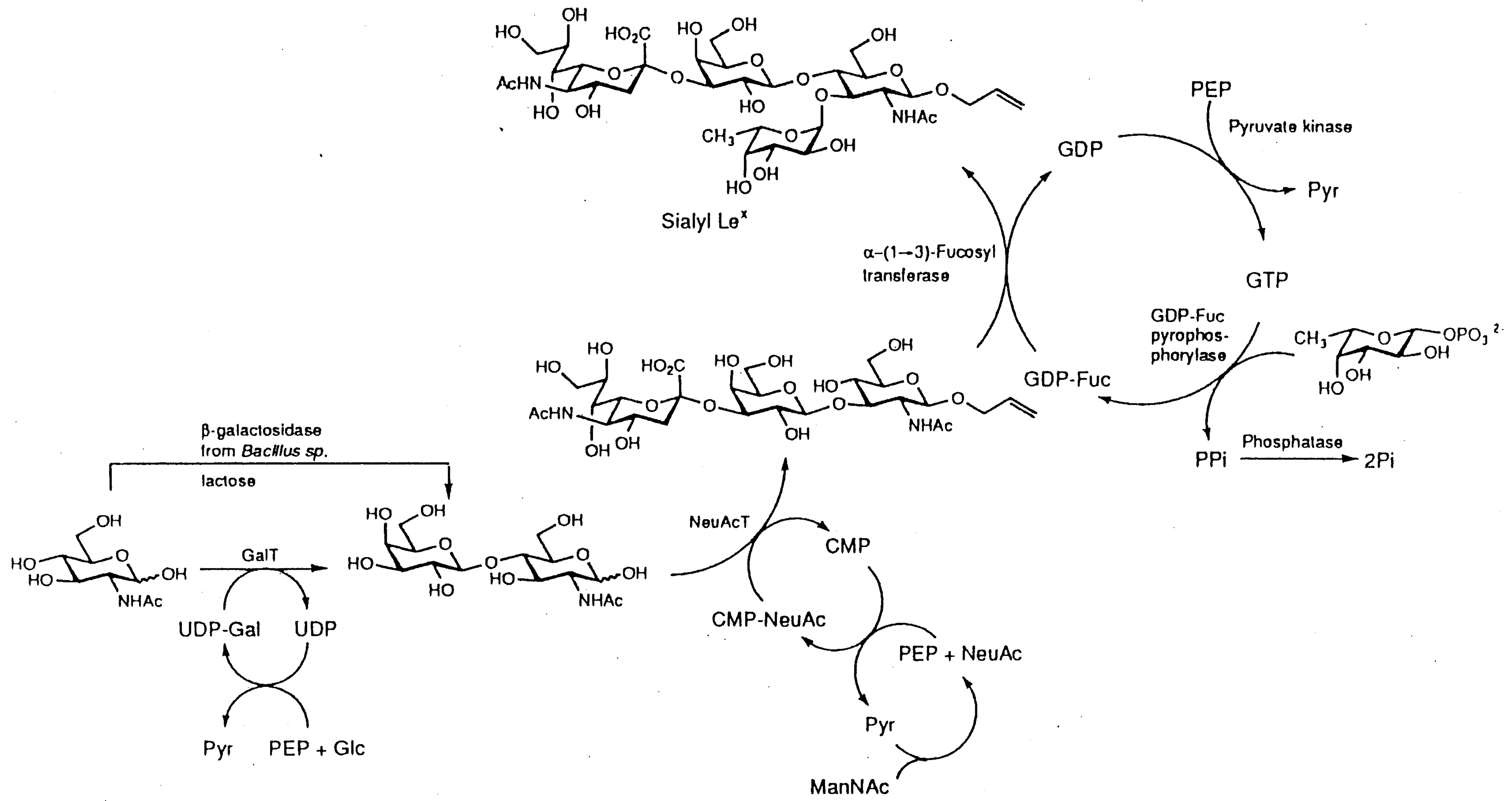
Glycosyl donor is a Sugar Phosphate instead and so the leaving group is just phosphate ion





E1: β-(1→4)-Galactosyltransferase; E2: Pyruvate kinase; E3: UDP-Glc pyrophosphorylase
E4: Galactose-1-phosphate uridyltransferase; E5: Galactokinase




Sialyl Le^x
PEP
Pyruvate kinase
Pyr
GDP
GTP
α-(1→3)-Fucosyl
transferase
GDP-Fuc
pyrophos-
phorylase
GDP-Fuc
PPi
Phosphatase
2Pi
β-galactosidase
from Bacillus sp.
lactose
GalT
UDP-Gal
UDP
Pyr
PEP + Glc
NeuAcT
CMP
CMP-NeuAc
PEP + NeuAc
Pyr
ManNAc